# Bioassays with bruchid beetles: problems and (some) solutions

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*Abstract*

Up to 20 species of seed beetle (Coleoptera: Bruchidae) have been described as pests of stored legume seeds. The most important, in an international context, are members of the genera *Callosobruchus*, *Acanthoscelides* and *Zabrotes*. Species of *Bruchus* are more frequently encountered in the field and pose less of a problem in storage situations.

There have been attempts in several organisations to breed cultivars of the primary legume crops susceptible to bruchid attack which provide some measure of resistance to the beetles. There are also numerous papers especially, but not exclusively, from developing countries describing surveys of local cultivars and selections for bruchid resistance. If any reports of bruchid resistance either among traditional varieties or new products of genetic engineering are to be worthwhile and compatible, then it is essential that the bioassays provide reliable information which is unambiguous and from which reliable conclusions can be drawn. Regrettably, this has not always been the case.What are the problems in designing effective bioassays using bruchids? Can they be overcome? If so, how?

## Bruchid Beetles as Pests

There are about 1300 species of seed beetle in the family Bruchidae. Of these about 20 are recognised as being pests, usually in stored legume seeds and especially in developing countries (Southgate 1979). Four species are of cosmopolitan importance. These are *Callosobruchus maculatus*, *C. chinensis*, *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. Other species of *Callosobruchus* including *C. analis*, *C. rhodesianus* and *C. subinnotatus* constitute a secondary group of storage pests, and *Bruchus pisorum*, *B. rufimanus* and *Bruchidius atrolineatus* are important as pests in the field and early stages of storage (Southgate 1978).

Most experimental work has concentrated on the four species of international importance since all of these species are responsible for significant losses from stores of seed held by subsistence farmers for food, marketing and as a source of seed for the next crop. Because of the nature of the situations in which these losses occur, accurate data on the scale of the damage are hard to find but frequently indicate that it exceeds 40% (Caswell 1981; Cardona and Karel 1990).

Even in countries, such as Australia, with access to conventional fumigant and chemical insecticides, the success of treatments has not always been established, and work on bruchid control is still in progress (e.g. Daglish et al. 1993).

## Control Measures for Bruchids

The essence of any control measure is to reduce biological fitness (Begon et al. 1990) which in this context, is usually measured simply as increased mortality. Actually it is not especially important which fitness parameters are affected. For example, increased larval mortality or decreased fecundity would both contribute to control of a pest population. Any means of decreasing fitness compatible with environmental safety, where the environment is taken to include people and their domestic stock, would be potentially acceptable.

In the context of their predominant importance as pests in developing countries, bruchid control is not a simple matter. Chemicals require a recurring expenditure and, for their safe use, appropriate levels of education. Biological control involving the application of predators, parasites or microbiological agents demands initial expenditure and, often, appropriate subsequent monitoring (Dent 1991). Neither funding nor expertise are always available. Cultural control is usually embodied within conventional traditional practices and may not readily be improved. Consequently, there is a major incentive to identify and use 'resistant' cultivars of the crops which may, in the longer term, provide a relatively cheap additional protection against losses. In some cases the identification of such cultivars has simply been based on a survey of crops already in use in different areas, and in others has involved systematic screening of a large germplasm bank and subsequent plant breeding (Singh 1978), the costs being borne largely, though not exclusively, by aid agencies. One assumes that the objective is to provide a self-sustaining and hence inexpensive control, although this assumption has already been questioned since insect resistance to the resistant factor(s) has already been demonstrated (Dick and Credland 1986b).

## Bruchid Beetles as Experimental Subjects in Bioassays

Whether studies of bruchids are for purely academic or for more practical reasons, much of the work demands a series of effective bioassays. However, the literature on bruchid control is composed of numerous inconsistent reports which frequently cannot be compared with each other in any worthwhile way. The explanation for this situation lies in the diversity of bioassays which have been employed and, sometimes, inadequate appreciation of the variables which must be controlled. If progress is to be made in bruchid control and results are to be applicable on a global rather than local scale, then an understanding of the problems, and ideally standardisation of protocol, must be more widespread than is currently found.

## A Brief and Simplified Theoretical Background

A bioassay is simply an alternative term for an experiment in which biological material is the test subject. There is almost always a comparative element in the experimental design; the effect of a treatment is determined by comparing the behaviour of the living material in the presence and absence of the treatment or by comparing the effects of a range of treatments of which at least one is presumed to be stable and

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'known'. In conventional terminology, some form of 'control' is an essential feature of the experimental design.

The bare essentials of any worthwhile bioassay are, like those of any other experiment, that it could be repeated by another scientist in another location. This presupposes that the original bioassay has stipulated variables and the other components are precisely defined and fixed. The only variable should be the treatment under investigation. The physical conditions of the assay should be regulated in an appropriate way and the subjects described appropriately.

In practice biological experiments can seldom, if ever, conform to this ideal because of the nature of the subjects and, sometimes, of the treatments. Biological material rarely approaches uniformity. Assays undertaken on clones or highly inbred strains of animals exhibiting minimal genetic variation and maintained under similar, strictly controlled conditions may be useful in some contexts, but the inherent variation between individuals is thereby deliberately minimised although it is a feature of almost all biological material. The variation of response is frequently as important as a repeated response elicited in a number of replicates. Bioassays should reveal this variability among the subjects as well as a 'normal' response. Furthermore, results determined in a series of tests on a single population, collection or culture of insects are almost invariably ascribed to the species although we know that in many cases there are major differences between populations (Diehl and Bush 1984). Here is a paradox; we demand that bioassays are repeatable but we know that our subjects, the test organisms, are inherently variable.

It is possible to apply materials to an insect in a very precise dose and measure many chemical parameters with great precision, although sometimes with less accuracy than is suggested (see Robertson and Preisler (1992) for an analysis and explanation of these problems). Thus, bioassays involving treatments with purified chemicals are *relatively* easy to standardise compared with research in which biological material constitutes the treatment as well as the subject. Where one is interested in the response of one sort of biological material to another, bioassays are much more complex and interpretation correspondingly more difficult.

The design of bioassays must include consideration of the physical conditions, the subjects, and the treatment. These three elements will now be considered separately, although this is an artificial division and there are complex interactions between them. No attempt is made to deal with the statistical basis of experimental design since Robertson and Preisler (1992) have already provided a comprehensive treatment of this issue in the context of pesticide bioassays with arthropods in general. The following sections are concerned only with bioassays involving pest species of bruchid in laboratory situations, particularly with reference to the determination of resistance among their host seeds, although the general principles are universally applicable.

## Physical Conditions

It has been known for at least 30 years that many environmental variables affect the fitness of bruchids. Howe and Currie (1964) showed that different temperatures and relative humidities had a very significant effect on the fecundity, developmental speed and longevity of several of the most important bruchid species. Southgate (1964) pointed out that *C. rhodesianus* was typically found in cooler areas than *C. maculatus*, and *Zabrotes subfasciatus* is a pest of *Phaseolus vulgaris* at lower altitudes (which are warmer and more humid) being replaced by *A. obtectus* at higher altitudes (Cardona and Karel 1990). One should therefore consider whether the performance of these pairs of species should be compared under the same physical conditions or under those in which they are normally found. Lighting regimes are often less important but in some cases are critical; *Bruchidius atrolineatus* will effectively stop breeding altogether in certain lighting regimes as it exhibits a facultative reproductive diapause (Lenga et al. 1991).

Some bruchids limit oviposition in the absence of an adequate supply of hosts. Females of some populations of *Callosobruchus maculatus* withhold eggs if provided with 10 seeds and lay significantly more on 40 or 100 (Credland 1986). Thus, realised fecundity (Mitchell 1990) is reduced although potential fecundity is maintained.

Some, perhaps all, bruchids will oviposit on a range of inappropriate substrates for reasons which are not fully understood. Many bruchids will attach eggs to the walls or floor of Petri dishes or glass tubes in experimental situations. This can occur even if surplus seeds which bear no eggs but appear indistinguishable from others bearing eggs are readily available. Therefore it is easy to underestimate fecundity and impossible to estimate the significance of a treatment applied to the beetles using this parameter without consideration of this potential problem.

In the case of *A. obtectus*, the proportion of eggs attached to seeds is immensely variable but no more than 50% are usually attached and many are easily dislodged (Quentin et al. 1991; D. Parsons and C. Moss, unpublished data). Although it is well known that larvae of this species move among seeds it is inevitable that fecundity measurements demand additional care. The variable fecundity figures quoted for this species may well be due to the omission of eggs not attached to seeds.

## The Subjects

### Inter and intraspecific variation among bruchids

Different species of bruchid typically occupy different habitats. Each species characteristically infests a different host although there is now a considerable measure of overlap among the major pest species (Southgate 1978). *A. obtectus* and *Z. subfasciatus* are usually found in stores of common bean, *P. vulgaris*, with which they are associated in South and Central America where they originated (Cardona and Karel 1990). Both species have now been found on beans and cowpeas, *Vigna unguiculata*, in Africa (Meik and Dobie 1986; Olubayo 1993). *C.maculatus*, the cowpea seed beetle, probably originated on cowpeas in West Africa but can now be found on chickpea (*Cicer arietinum*), adzuki bean (*Vigna angularis*), lentils (*Lens culinaris*) and numerous other legume seeds. *C. chinensis*, the adzuki bean weevil, probably evolved in Southeast Asia in association with a wild progenitor of the adzuki bean (Yoshida et al. 1986), but has now spread onto numerous alternative hosts. Thus there are major differences in host utilisation and although the pest species are more polyphagous than their wild relatives, there are limits to the host range of each species (Credland 1990); for example, *C. maculatus* cannot reproduce on *P. vulgaris* (Janzen et al. 1976).

The fact that Southgate (1964) could identify *C. rhodesianus* as more typical of cooler areas than *C. maculatuss*, epitomises the differences in physical conditions to which even closely related species are adapted. Therefore if one is comparing species, the physical conditions employed are of paramount importance. One needs to decide if the objective of an assay is to compare performance under a single or multiple set of conditions, or to determine the limit of each species' performance under optimal or marginal conditions.

Interspecific differences are probably less of a concern, since they are more readily recognised, than intraspecific differences. The very fact that the species have different names indicates a measure of difference even if it is not always defined. Intraspecific differences are much more difficult to quantify and are frequently not even recognised, although there has been a greater appreciation of the problem in the last few years (Credland 1990). Fujii (1968, 1969) showed that differences existed among populations of bruchid species nearly 30 years ago, but the practical implications have only become apparent more recently (e.g. Credland 1990; Credland and Dendy 1992). In these and other studies major differences are expressed between geographical biotypes (Diehl and Bush 1984) in parameters such as fecundity and developmental speed.

One of the simplest demonstrations of the differences between populations can be provided by looking at susceptibility to an insecticide (in one laboratory), since physical conditions and treatment are identical in each assay. Exposing adults of five populations of *C. maculatus*, maintained without exposure to any insecticide for at least 150 generations, to malathion immediately exposes variation (Fig. 1). It is therefore not surprising that the capacity to survive in so-called 'resistant' seeds should also be exhibited (Dick and Credland 1986a; Giga et al. 1993). Separate populations respond differently in the same bioassay, although the scale of the differences vary with the nature of the assay. It should also be obvious that averaging results of assays in different laboratories, probably using different biotypes, has no biological meaning, being unrepresentative of the species or, perhaps, any part thereof.

Bioassays conducted on a single biotype do not represent the response of that bruchid species to a treatment.

## Individual variation

Hidden within the previous argument is the assumption that individual insects within the population differ, and that differences can be overcome with appropriate statistical tests or manipulations (Robertson and Preisler 1992). This is probably the case in many situations where appropriate attention is applied to other parameters of the bioassay, such as the physical conditions, but the extent of individual variation is frequently extreme and unpredictable.

One major problem is the incidence of polymorphism in *C.maculatus* (Utida 1981) which is primarily induced by elevated seed water content (Sano-Fujii 1984) but also has a genetic basis (Sano-Fujii 1986; Messina 1987). A comparable problem may occur in other species (Credland 1990). Most authors have worked in conditions which ensure production of the normal (nonflight) form which reproduces immediately after adult emergence and has a higher fecundity than the active or flight form (Utida 1972). The extent of individual variation under tightly controlled conditions can be illustrated by reference to recent studies on *Acanthoscelides obtectus* (Parsons and Credland, in prep.). In my laboratory this species exhibits greater individual variation within a population, under regulated physical conditions, than any other bruchid on which we have worked. For example, most bruchids which have been studied exhibit a predictable pattern of oviposition activity under specified conditions. All the biotypes of *C. maculatus* which have been studied lay most of their eggs on the first day after emergence under optimal or near-optimal physical conditions (Dick and Credland 1984). The number of eggs laid on each succeeding day declines so that virtually all have been laid by the sixth day after emergence (see Credland and Wright 1989, for details). In the case of *Zabrotes subfasciatus* most eggs are usually laid on day 2 (Howe and Currie 1964; Meik and Dobie 1986). However, in the case of *A. obtectus* there is immense variation and although an overall pattern such as that in Figure 2a can be obtained, it is a composite of numerous diverse patterns such as those in Figure 2b which represent the daily oviposition of six individual females. Full sibs may display all these and other patterns, regardless of that displayed by their mother or paternal aunts. Whether the patterns have a genetic basis or represent the product of complex environmental interactions is unknown.

In many cases, it can be shown that statistically significant variation in parameters such as adult weight or fecundity has an environmental basis and, hence, can be manipulated by the physical conditions to which animals were exposed before or during the bioassay. A simple example of the situation where inattention to detail can produce wide fluctuations in parameters frequently used in bioassays, is the association between

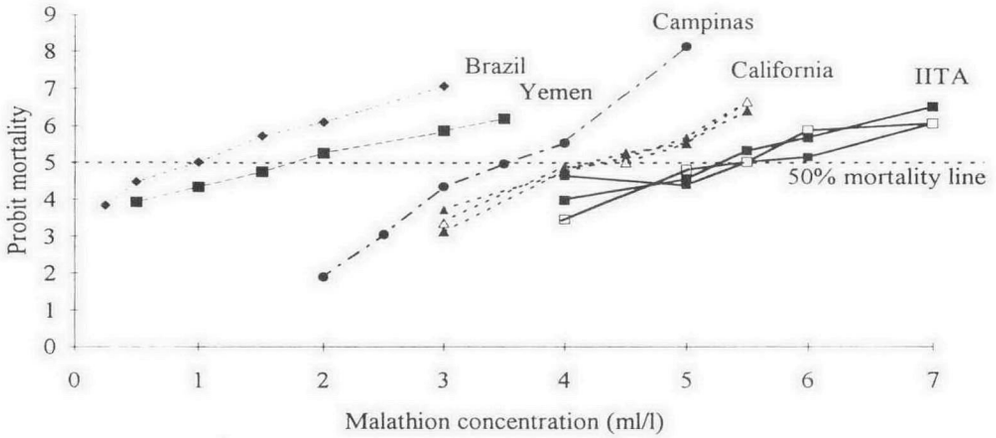


**Fig. 1.** Probit mortality of five populations of *Callosobruchus maculatus* exposed to a commercial emulsifiable concentrate of malathion. Each point represents the mean of 9 replicates of 10 adult beetles which were confined on 5 $cm^2$ of filter paper to which the specified concentration had been applied. Mortality was assessed after 10 hours exposure at 25°C. Three entirely separate assays were undertaken for the California and IITA populations, and one for each of the others. 1 mL/L malathion corresponds to exposure to 0.0031 mL of technically pure active material.

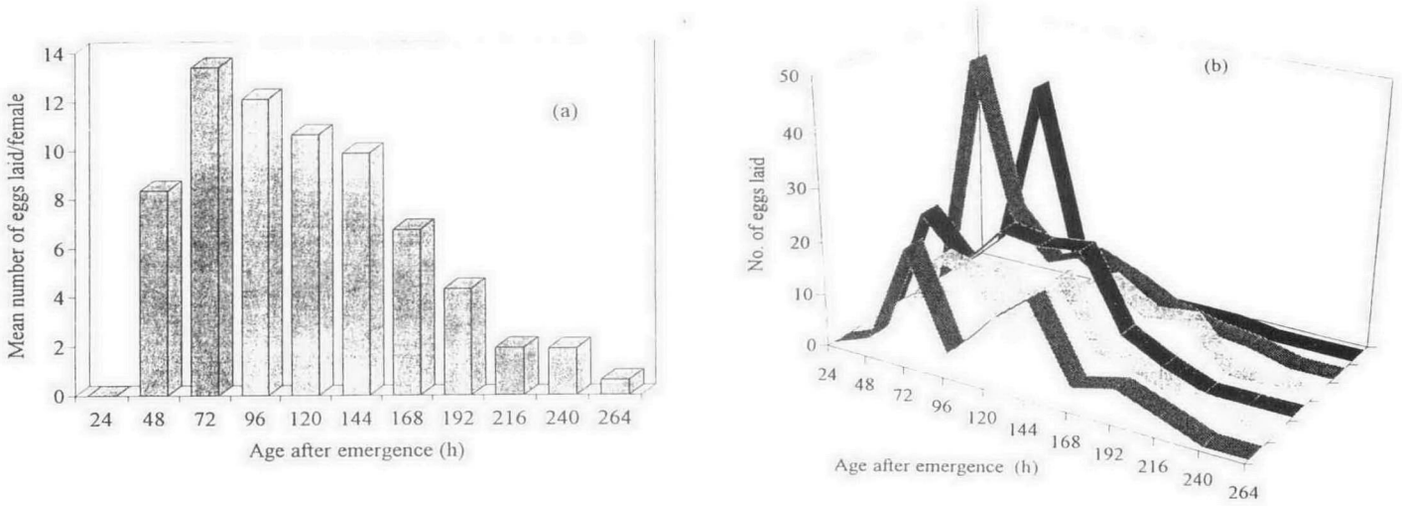


**Fig. 2.** Daily oviposition by female *Acanthoscelides obtectus* reared at a uniform density and provided with four fresh, conditioned seeds per day. Females were allowed to mate during the initial 24 hours and put on beans after this period. (a) Mean results from 101 individual females. (b) Results for six individual females to show the immense variation which occurs even under rigorously standardised conditions.

larval density within a seed, the number and weight of adult insects which emerge and the fecundity of the females among them (Credland et al. 1986; Desroches 1990).

It is now well established that increasing larval density within a seed leads, not surprisingly, to a greater number of adults emerging in many populations of some bruchids such as *C.maculatus* and *C. chinensis*. However, the increase is not linear and competitive interactions produce a plateau representing the maximum number of individuals which can successfully complete their development in a seed of given size (Bellows 1982a, b; Smith and Lessells 1985). The larvae of some populations, such as the South India biotype of *C. maculatus*, are highly competitive and it is rare for more than a single adult to emerge from any seed (Mitchell 1991); this represents an interesting extreme case of the differences noted among populations by Dick and Credland (1984). There are also interspecific differences since all recorded *C. analis* larvae appear to be competitive and it is rare for more than a single adult to emerge from any seed (Toquenaga and Fujii 1990). Therefore, survival of individual larvae is a factor not only of their own viability in the context of their host and physical conditions, but also of the number of other larvae within the seed that they occupy. Where multiple larvae do survive in a single seed, then their weight is often significantly smaller as the larval density increases (e.g. Credland et al. 1986). Thus, increasing larval survival can result in higher densities and smaller body weights, the former suggesting an increase in fitness and the latter, a decrease. However, neither may reveal anything about the chemistry or physical attributes of the seed since the results can be a consequence of space and individual behaviour. Female fecundity in *C. maculatus* increases with body weight and there is no independent role of larval density (Colegrave 1993). Therefore, like body weight, fecundity of insects is markedly affected by larval density.

These examples illustrate that experimental protocols which fail to regulate larval density can produce a mass of data which are so interwoven that it is virtually impossible to make a sensible interpretation of their meaning. Nevertheless, perhaps the most common assays have involved introducing 'x' insects to 'y' seeds and measuring precisely those parameters of individual insects which are subject to modification by the unregulated larval density.

## Treatments

If the physical conditions and protocol of the bioassay and the inherent variation in the living material, the bruchid subject, can be recognised, controlled and their effects thereby limited, the remaining variable(s) is the treatment applied.

In those cases where the treatment is 'simple', the assay, for example, of a predetermined dose of a pure chemical formulated in a defined way and applied in a standard manner, there are no problems which differ from those which impinge on routine assays undertaken in numerous laboratories every day. Concerns over purity, degradation, losses during application, the definition of incapacity or death in a practical context, do not require any abnormal attention in the context of bruchid beetles. The same criteria for rigour which apply to all insecticide assays should be employed (Robertson and Preisler 1992).

The problems are much greater in assays which involve botanical material and especially whole seeds. The search for bruchid resistance in seeds has always depended on, and continues to depend on, exposing seeds to beetles and observing parameters such as oviposition, development speed and larval survival (CIAT 1986). Subsequently reports usually cite a germplasm bank or cultivar name or number and regard this as a constant in the experimental analysis. Unfortunately, this is inappropriate and can sometimes be misleading.

Perhaps the simplest problem to understand is the source of the material. In the great majority of cases, the bioassays are not conducted by the collector or breeder of the seed. If the seed has been collected from the field, it is imperative that the material is homogeneous, and not a heterogeneous mix of seeds, ideally from an individual plant and certainly from a single stand or area. Frequently it is unknown if this is the case and certainly it is rarely stated in the published accounts. While different plant species may possess characteristic cocktails of allelochemicals (Liener 1982), it is well known that the relative concentrations of the components of the cocktails and the absolute levels of each component can vary for both genetic and environmental reasons (Zangerl and Berenbaum 1993). For example, chemical composition may vary with soil characteristics and exposure.

Whilst it is unreal to imagine that complete analyses of each seed tested or even a representative sample could be undertaken in every bioassay, a simple account of the source of the

seed, its consistency in appearance and, if it were bred, its pedigree would be an invaluable adjunct to the data presented.

In addition to any chemical differences between seeds, the physical differences between accessions or cultivars can have far reaching consequences in terms of resistance measurement. Size alone can determine the number of bruchids which emerge, so that comparative assays in which seed number is constant can be misleading if larval density is not controlled. Conversely, if seed weight is kept constant then the number of seeds may vary considerably. Some bruchids distribute their eggs uniformly among the available seeds and only a single adult emerges from each seed (Thanthianga and Mitchell 1990). Seed number will then be more important than seed weight in determining the number of progeny completing development.

The physical characteristics of the seed testa can determine acceptability for oviposition but may not be related to the antibiotic nature of the seed (Messina and Renwick 1985). Nwanze et al. (1975) showed that rough seeds were less acceptable to *C. maculatus* than smooth ones. If no account is taken of differential oviposition, then it would be easy to attribute resistance to seeds, measured as adult emergence, on the basis of their acceptability for oviposition. (While it is true that reduced acceptability could potentially be used to contribute to resistance, it is important to be able to segregate behavioural and physiological resistance at an early stage in screening.)

Seed shape is seldom mentioned in bioassays with bruchids but is critically important. For example, some wild accessions of *Phaseolus vulgaris* are flattened and reniform but mungbeans (*Vigna radiata*) are almost spherical. The larvae of *Acanthoscelides* move from one seed to another before penetrating the testa. They need to be able to bridge a gap somewhat shorter than their bodylength to provide a brace to maintain their position whilst excavating the early stages of a penetration hole. Rounded seeds generally provide fewer such points (Quentin 1991) and any movement of the seed disrupts boring and can prevent penetration, so providing a physical means of their control (Quentin et al. 1991). In many assays, especially if the timing of penetration is to be measured precisely, so requiring regular examination of the seeds, then the movement of the seeds within their containers is enough to inhibit penetration and false resistance may be recorded (C. Moss, per. comm. 1993).

Among other seed differences which may influence the outcome of bioassays but have seldom been considered methodically, are the hardness of the testa (Thiery 1984), its softening on exposure to air with different water content, its age (since some of its constituent chemicals may degrade thereby producing or destroying toxins), and the means of its storage which can affect any or all of these parameters. Furthermore, the effects of any microorganisms which may be associated with the seed, especially in damp conditions, need to be considered.

## Some Pragmatic Solutions

Many of the problems which have been mentioned in the context of bruchids are found and have probably been solved in work with many other pests. Unfortunately these solutions, if they exist, have not permeated very far into the world of bruchid biology and perhaps this provides some explanation of why many basic problems remain and why so much work stands in isolation and cannot be integrated into more helpful solutions of the important practical problems which face us today (Singh 1990). In a database of over 1000 publications, excluding purely taxonomic works, on bruchids accumulated over the last 12 years, there is not a single paper which provides genuine, reliable *data* on losses that they cause in the field or store anywhere in the world. There are numerous assertions, estimates and guesses. Literally dozens of papers deal with 'resistance' in numerous hosts, cultivars and accessions, but there is no pattern among the data and it is impossible to compare almost any one paper with any other. The preceding sections explain, in part, how this latter situation has arisen. Why it has arisen is more contentious but may have much to do with the availability of literature in those areas where most of the bruchid problems occur and the understandable demand for quick local solutions rather than an aspiration to broaden the basis of our fundamental knowledge. Nevertheless, the consequence is that much work which could be important is overlooked and there can be no doubt that a wider dissemination of knowledge and experience would be valuable. The scientific community will be more receptive when the work has a more structured basis and it is apparent that the data have been collected in a rigorous way which excludes or at least recognises the variables to which reference has been made. What is required? What practical steps can be taken to alleviate if not solve the problems?

In terms of the physical conditions, it should be a minimum requirement that conditions are defined and controlled. Ambient conditions permit such variation in insect performance (fitness) that very limited value can be ascribed to data obtained under such conditions. The bioassay could hardly be repeated in the same laboratory under the stability of some tropical conditions, and certainly never be copied elsewhere. Temperature, relative humidity and the lighting regime need to be defined with means and the range. It is important that they are actually measured rather than set since, for example, even an open dish of water which is continually replenished will not maintain humidities above about 30% in some incubators (P. Credland, unpublished data). Depending on the objective of the study and the species concerned, there is no reason why these three variables should be constant throughout the day; some diurnal cycling may be desirable or essential but requires definition. The actual values which are preset will usually be determined in the context of either those experienced by the species in its normal local habitat, those which produce maximum fitness (if they have been defined) or those under which it competes with other species. An explanation of the values selected, in the preceding terms, would always be desirable.

The capacity of many bruchids to lay on 'inappropriate' surfaces needs to be taken into account. It can be minimised or avoided altogether by enclosing seeds in containers lined with coarse emery paper on which oviposition has never been seen in, literally, thousands of individual cases (Dick and Credland 1984). If smooth glass or plastic containers are used, then checks for oviposition on these surfaces are essential. These considerations do not apply to *Acanthoscelides* in which the larvae are motile and can move among seeds but care must be taken in counting the eggs.

The subjects of the experiments, the insects, must be described in unambiguous terms. If it is intended that the results of the assays should be applicable to a species, then recognition of 'the biotype problem' is a minimal requirement (Diehl and Bush 1984). No single population can represent the diversity expressed by an entire cosmopolitan species (Credland 1990); this is not because the species has no defining characteristics but is because responses, both terminal and intermediate, may include steps or make metabolic demands on characteristics which do show intraspecific variation. Therefore, while a purely local problem needs to be approached with reference to its own endemic bruchid population, one cannot extrapolate from these results to a different locality or to the species as a whole.

Preceding any bioassay it is imperative that the insects to be employed are reared at a density of one insect per seed to minimise variation due to polymorphism and standardise their size and potential fecundity (Mitchell 1990). However, it is important that the range of responses as well as the 'typical' response of the population is recognised. Environmentally induced variation can arise even in regulated physical conditions as explained previously. The adult density on seeds to be tested and larval density within the seeds need careful regulation since both affect many of the parameters usually used in bioassays (e.g. Padgham et al. 1992; Schoonhoven et al. 1983; Simmonds et al. 1989). With unlimited seed, maintaining a larval density of one per seed is to be preferred since this excludes any variation in competitiveness within the species. Furthermore, infestation with single larvae can be established in very small seeds which are too small to allow the production of two adults for physical reasons. The uniformity in size among adults which develop individually is greater than among adults developing at variable and unknown densities, permitting more precise comparisons between hosts to be made (B. Tran and P. Credland, unpublished data). Individual variation among the progeny can then be accommodated by the use of statistical methods of the kind explained by Robertson and Preisler (1992).

If larval density is to be regulated, one obvious sequitur is that oviposition and subsequent assays need to be conducted independently. If this is not done then increasing egg number may lead to an increase in density (hatched eggs/seed), thence smaller, less fecund adults, and, possibly, more rapid development (Credland et al. 1986). Thus a change in one parameter can lead to correlated changes in others, producing spurious or misleading results. The combined, accurate, measurement of oviposition on the seed and subsequent measurement of suitability for development can only compromise each other to the extent that neither measurement has any value. Bearing in mind that many bruchids will oviposit on an enormous range of surfaces, if seed is limited it is generally preferable to measure aspects of development and mortality with precision. Except in the case of *A. obtectus*, some oviposition on the seed must occur for this experiment to be undertaken and therefore some degree of acceptability has been demonstrated.

The final factor to be considered is the nature of the seed itself. In the determination of any differential resistance among seeds, the quantity of homogeneous seed which is available usually delimits the experimental protocol. The size of mature seeds is a characteristic of the cultivar or wild form, not of the species. Red kidney beans may weigh 500 mg and be 17 mm long compared with some wild accessions of the same species, *Phaseolus vulgaris*, whose seeds weigh only 30 mg and are 5 mm long (C. Moss, unpublished data). It is impossible to generalise about the treatment of this variable. For example, if larvae are not competitive and the individual seeds are large then it may be possible by removal of excess eggs and ensuring separation of eggs on each seed to exploit the seed size by allowing a small, known number of larvae to penetrate and, perhaps develop. (Single infestation is still to be preferred, if possible.) Conversely, to undertake assays with more than one egg on some of the very small, flattened seeds of wild *P. vulgaris* is wasteful since no more than one adult will develop for size reasons alone. The proportion of eggs producing an adult can be manipulated in such situations by simply changing the egg: seed ratio. Seed shape also needs to be considered; if seeds are spherical then oviposition by those species which glue their eggs to the seeds and penetration by larvae of *Acanthoscelides* are more difficult. These problems are minimised if more than one seed is used to provide additional contact points and the seed(s) fit closely into the container. If seeds are in short supply, clean glass beads of comparable size to the seeds may provide a suitable alternative.

Seed conditioning is an essential prerequisite. With large (5–6 mm) seeds such as cowpeas, it takes at least 2 weeks in the experimental conditions for the moisture content to reach equilibrium (Dick and Credland 1984). Since seed water content can determine the morph of the adult *C. maculatus*, which are characterised by different fecundities (Sano-Fujii 1984; Ouedraogo et al. 1991), the importance of this period cannot be overestimated. Measurements of seed water content should be made routinely. Seed age is a parameter which has not been investigated systematically but is a potential problem. Since the purpose of most experiments is to investigate seeds that will typically be stored for less than a year, naturally dried, mature seeds harvested within the past year should be used when possible.

## Conclusion

The object of this contribution was to try and expose the problems in undertaking measurement of bruchid resistance in seeds. The difficulties can be divided into three areas, although interactions between them result in making a clean division impossible. Some of the problems can be overcome by careful consideration of experimental design including pretreatment of the test organisms and the biological material (seeds) under investigation. By unifying design of bioassays, or at least recognising the problems to which attention has been drawn and providing adequate descriptions of conditions and data, far greater interchange of information and more rapid progress will be made. The difficulties which have been highlighted do not constitute a reason for hopelessness or despair; they can all be overcome or mediated. It is a fact that biotypical differences may ultimately prevent the pronouncement of *simple* answers referable to a whole species, but if the problem is appreciated then satisfactory progress can be made with even the most cosmopolitan pest species. Total uniformity in experimental design may be too much to expect and perhaps undesirable in some respects, but a widespread appreciation that experiments should be repeatable and that accounts should provide all the information to enable this to happen would be an invaluable advance.

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